SARCOMA 180 (CODE 3SA)

S180: Implanted subcutaneously in axillary region of Swiss mice. Treatment begun 24 hours after implant. One dose daily for 7 days. Animals sacrificed on 8th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: Noninbred albino mice (Swiss).
- Weight range: Male and Female: 18–22 grams $\bar{x} \geqq 19.5$ grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Six animals per test group (Sec. 9.101).
- Assay testing: Four animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region by trocar.
- Size of implant: Average diameter 2–4 mm per fragment.
- Propagation: For line, 7 day; donors for test, 6–7 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered *control groups. Record survivors daily.*
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 7.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 8: Sacrifice and record wt. of animals and tumors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 8, and test/control (T/C) of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 500–2000 mg. Compute standard deviation (S.D.) of mean (Sec. 11.003). Positive control limit $\leqq 42\%$. For NSC-3051, use 200 mg/kg/day. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final (Day 8): Prepare final control and test reports. Send for key-punching.

CRITERIA: *(Protocol 12)*

Sequential: (Sec. 12.101)

	Stage 1	Stage 2	Stage 3
Synthetics	$\leqq .53$	$\leqq .19$	$\leqq .07$
Natural products	$\leqq .44$	$\leqq .19$	

Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201).
Natural products: 2 successive dose-response tests (Sec. 12.204).

ADENOCARCINOMA 755 (CODE 3CA)

Ca755: Implanted subcutaneously in axillary region of BDF_1 mice. Treatment begun 24 hours after implant. One dose daily for 11 days. Animals sacrificed on 12th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: BDF_1 (C57BL/6 × DBA/2)F_1, other suitable hybrid or C57BL/6 mice.
- Weight range: Male: 18–22 grams $\bar{x} \geqq 19.5$ grams; Female: 17–21 grams $\bar{x} \geqq 18.5$ grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Ten animals per test group (Sec. 9.102).
- Assay testing: Six animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region by trocar.
- Size of implant: Average diameter 2–4 mm per fragment.
- Propagation: For line, 14 day; donors for test, 12–14 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 11.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 6: Prepare fresh compound for subsequent testing.
- Day 12: Sacrifice and record wt. of animals and tumors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 12, and T/C of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 500–2000 mg. Compute S.D. of mean (Sec. 11.003). Positive control limit $\leqq 42\%$. For NSC-749, use 50 mg/kg/day. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final (Day 12): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)
- Sequential: (Sec. 12.101)

	Stage 1	Stage 2	Stage 3
Synthetics	$\leqq .53$	$\leqq .19$	$\leqq .07$
Natural products	$\leqq .44$	$\leqq .19$	

- Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201). Natural products: 2 successive dose-response tests (Sec. 12.204).

LYMPHOID LEUKEMIA L1210 (CODE 3LE)

L1210: Ascitic fluid implanted intraperitoneally in BDF_1 mice. Treatment begun 24 hours after implant. One dose daily until death. Mean survival time of test animals compared with that of control animals. Results expressed as percent of control survival time.

ANIMALS: (Protocol 8)
- Strain: BDF_1 ($C57BL/6 \times DBA/2)F_1$, other suitable F_1 hybrid, or DBA/2 mice.
- Weight range: Male: 18–22 grams $\bar{x} \geqq 19.5$ grams; Female: 17–21 grams $\bar{x} \geqq 18.5$ grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Six animals per test group (Sec. 9.101).
- Assay testing: Four animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Inject intraperitoneally.
- Size of implant: 0.1 ml diluted ascitic fluid containing 10^5 cells.
- Propagation: For line, 7 day; donors for test, 6–7 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 until death.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 5: Weigh animals and record.
- Day 6, 13, & 20: Prepare fresh compound for subsequent testing.
- Day 30: Sacrifice all survivors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 5, and T/C of mean survival time for all groups with more than 65% survivors on Day 5.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control survival: 8–11 days. Compute S.D. of mean (Sec. 11.102). Positive control limit $\geqq 135\%$. For NSC-749, use 50 mg/kg/day. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final day: Prepare final control and test reports on day of last animal death. Send for key-punching.

CRITERIA: (Protocol 12)
- Sequential: (Sec. 12.103)

	Stage 1	Stage 2
Synthetics	$\geqq 1.25$	$\geqq 1.56$
Natural products	$\geqq 1.25$	$\geqq 1.56$

- Confirmation: Synthetics: Dose response with T/C of at least one dose $\geqq 125\%$ (Sec. 12.202). Natural products: Two successive dose-response tests (Sec. 12.204).

L1210/MTX (METHOTREXATE-RESISTANT) (CODE 3LX)

LX: Ascitic fluid implanted intraperitoneally in BDF_1 mice. Treatment begun 24 hours after implant. One dose daily until death. Mean survival time of test animals compared with that of control animals. Results expressed as percent of control survival time.

ANIMALS: (Protocol 8)
- Strain: BDF_1 ($C57BL/6 \times DBA/2)F_1$, other suitable F_1 hybrid, or DBA/2 mice.
- Weight range: Males: 18–22 grams $\bar{x} \geqq 19.5$ grams; Females: 17–21 grams $\bar{x} \geqq 18.5$ grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Six animals per test group (Sec. 9.101).
- Assay testing: Four animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Inject intraperitoneally.
- Size of implant: 0.1 ml diluted ascitic fluid containing 10^6 cells.
- Propagation: For line, 7 day; donors for test, 6–7 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive and negative control on all control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 until death.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 5: Weigh animals, and record.
- Day 6, 13, & 20: Prepare fresh compound for subsequent testing.
- Day 30: Sacrifice all survivors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 6, and T/C of mean survival time for all groups with more than 65% survivors on day 5.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control survival: 8–11 days. Compute S.D. of mean (Sec. 11.102). Positive control limit not established. For NSC-26271, use 25 mg/kg. Negative control limit not established. For NSC-740, use 0.75 mg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final day: Prepare final control and test reports on day of last animal death. Send for key-punching.

CRITERIA: (Protocol 12)
- Sequential: (Sec. 12.103)

	Stage 1	Stage 2
Synthetics	$\geqq 1.25$	$\geqq 1.56$
Natural products	$\geqq 1.25$	$\geqq 1.56$

- Confirmation: Synthetics: Dose-response T/C of at least one dose must be $\geqq 125\%$ (Sec. 12.204).
Natural products: Two successive dose-response tests (Sec. 12.204).

LEWIS LUNG CARCINOMA (CODE 3LL)

LL: Implanted subcutaneously in axillary region of BDF_1 mice. Treatment begun 24 hours after implant. One dose daily for 11 days. Animals sacrificed on 12th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: BDF_1 ($C57BL/6 \times DBA/2)F_1$, other suitable F_1 hybrid, or C57BL/6 mice.
- Weight range: Males: 18–22 grams $\bar{x} \geqq 19.5$ grams; Females: 17–21 grams $\bar{x} \geqq 18.5$ grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Six animals per test group (Sec. 9.101).
- Assay testing: Four animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region by trocar.
- Size of implant: Average diameter 2–4 mm per fragment.
- Propagation: For line, 14 day; donors for test, 12 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 7.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 6: Prepare fresh compound for subsequent testing.
- Day 12: Sacrifice and record wt. of animals and tumors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 12, and T/C for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 500–2000 mg. Compute S.D. of mean (Sec. 11.003). Positive control limit not established. For NSC-26271 use 25 mg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final (Day 12): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)

Sequential: (Sec. 12.101)

	Stage 1	Stage 2	Stage 3
Synthetics	$\leqq .53$	$\leqq .19$	$\leqq .07$
Natural products	$\leqq .44$	$\leqq .19$	

Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201).
Natural products: 2 successive dose-response tests (Sec. 12.204).

SOLID FRIEND VIRUS LEUKEMIA (CODE 3FV)

FVL: Implanted subcutaneously in axillary region of BDF_1 or Swiss mice. Treatment begun 24 hours after implant. One dose daily for 11 days. Animals sacrificed on 12th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: BDF_1 (C57BL/6 × DBA/2)F_1, other suitable F_1 hybrid, DBA/2, or Swiss mice.
- Weight range: Males: 18–22 grams $\bar{x} \geqq 19.5$ grams; Females: 17–21 grams $\bar{x} \geqq 18.5$ grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Ten animals per test group (Sec. 9.102).
- Assay testing: Six animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region by trocar or suitable brei.
- Size of implant: Average diameter of trocar 2–4 mm per fragment.
- Propagation: For line, 14 day; donors for test, 13–14 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 11.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 6: Prepare fresh compound for subsequent testing.
- Day 12: Sacrifice and record wt. of animals and tumors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 12, and T/C of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 500–2000 mg. Compute S.D. of mean (Sec. 11.003). Positive control limit not established. For NSC-26271, use 25 mg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final (Day 12): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)
- Sequential: (Sec. 12.102)

	Stage 1	Stage 2	Stage 3
Synthetics	≦ .63	≦ .23	≦ .08
Natural products	≦ .60	≦ .22	

- Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201).
  Natural products: 2 successive dose-response tests (Sec. 12.204).

CLOUDMAN MELANOMA S91 (CODE 391)

S91: Implanted subcutaneously in axillary region of BDF_1 mice. Treatment begun 24 hours after implant. One dose daily for 11 days. Animals sacrificed on 21st day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: BDF_1 (C57BL/6 × DBA/2)F_1 mice.
- Weight range: Males: 18–22 grams $\bar{x} \geqq 19.5$ grams. Females: 17–21 grams $\bar{x} \geqq 18.5$ grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Ten animals per test group (Sec. 9.102).
- Assay testing: Six animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region.
- Size of implant: 0.2 ml of 50% tumor suspension in saline.
- Propagation: For line, 21 day; donors for test, 21 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 11.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 6: Prepare fresh compound for subsequent testing.
- Day 12: Weigh animals. Record mean wt. under "Comments."
- Day 21: Sacrifice and record wt. of animals and tumors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 21, and T/C of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 500–2000 mg. Compute S.D. of mean (Sec. 11.003). Positive control limit not established. For NSC-26271, use 25 mg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final (Day 21): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)

Sequential: (Sec. 12.102)

	Stage 1	Stage 2	Stage 3
Synthetics	$\leqq .63$	$\leqq .23$	$\leqq .08$
Natural products	$\leqq .60$	$\leqq .22$	

Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201).
Natural products: 2 successive dose-response tests (Sec. 12.204).

OSTEOGENIC SARCOMA HE10734 (CODE 30S)

OS: Implanted subcutaneously in C3H/He mice. Treatment begun 10 days after implant. One dose daily for 10 days. Animals sacrificed on 20th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: Inbred C3H/He mice (or hybrids, if suitable).
- Weight range: Male and Female: 18–22 grams $\bar{x} \geqq 19.5$ grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Six animals per test group (Sec. 9.101).
- Assay testing: Four animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Subcutaneously by trocar.
- Size of implant: Average diameter 4 mm per fragment.
- Propagation: For line, 20–21 day; donors for test, 18–20 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Run positive control on odd-numbered control groups.
- Day 1 and 2: Check cultures; discard contaminated groups.
- Day 9: Determine solubilities; thaw solutions. Prepare materials.
- Day 10: Weigh and randomize animals (Protocol 10). Treat Day 10 through Day 19. Record survivors daily.
- Day 15: Prepare fresh compound for subsequent testing.
- Day 20: Sacrifice and record wt. of animals and tumors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 10, Day 20, and T/C of tumor wt. for all treated groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 500–2000 mg. Compute S.D. of mean control tumor wt. (Sec. 11.003). Positive control limit not established. For NSC-3138, use 1000 mg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 10): Mail initial control and test reports to Documentation Inc.
- Final (Day 20): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)

Sequential: (Sec. 12.101)

	Stage 1	Stage 2	Stage 3
Synthetics	$\leqq .53$	$\leqq .19$	$\leqq .07$
Natural products	$\leqq .44$	$\leqq .19$	

Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201).
Natural products: 2 successive dose-response tests (Sec. 12.204).

HEPATOMA 129 (CODE 3HE)

HE: Implanted subcutaneously in axillary region of C3H/He mice. Treatment begun 5 days after implant. One dose daily for 10 days. Animals sacrificed on 15th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: Inbred C3H/He mice (or hybrids, if suitable).
- Weight range: Male and Female: 18–22 grams $\bar{x} \geqq 19.5$ grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Six animals per test group (Sec. 9.101).
- Assay testing: Four animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region by trocar.
- Size of implant: Average diameter approximately 2–4 mm per fragment.
- Propagation: For line, 14 day; donors for test, 14 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Run positive control on odd-numbered control groups.
- Day 1 and 2: *Check cultures for contamination; discard contaminated groups.*
- Day 4: Determine solubilities; thaw solutions. Prepare materials.
- Day 5: Weigh and randomize animals (Protocol 10). Treat Day 5 through Day 14. Record survivors daily.
- Day 9: Prepare fresh compound for subsequent testing.
- Day 15: Sacrifice and record wt. of animals and tumors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 5, Day 15, and T/C of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 500–2000 mg. Compute S.D. of mean (Sec. 11.003). Positive control limit not established. For NSC–3051, use 200 mg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 5): Mail initial control and test reports to Documentation Inc.
- Final (Day 15): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)
- Sequential: (Sec. 12.101)

	Stage 1	Stage 2	Stage 3
Synthetics	$\leqq .53$	$\leqq .19$	$\leqq .07$
Natural products	$\leqq .44$	$\leqq .19$	

- Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201). Natural products: 2 successive dose-response tests (Sec. 12.204).

TOXICITY TEST IN RATS (CODE 5AA)
ALKYLATING AGENTS ONLY

AA: Three non-tumor-bearing rats given injections at each of four dose levels once daily for 5 days. Survivors at 10 days recorded. LD10 determined from log-probit plot.

ANIMALS: (Protocol 8)
- Strain: Suitable noninbred albino rats.
- Weight range: Male and Female: 90–110 grams.
- Sex; source: One sex and source for each compound.

EXPERIMENT SIZE: Three animals per dose.

TESTING SCHEDULE: Prepare compound fresh daily.
- Day 1–5: Inject one dose daily, at four doses: 100, 33, 10, 3 mg/kg. Record survivors daily.
- Day 6–10: Record survivors daily.

EVALUATION: Plot survivors at 10 days versus dose, on log-probit graph paper. Estimate LD10. If LD10 <3 mg/kg, repeat test at dose of 3, 1, 0.3, 0.1 mg/kg. Do not repeat if LD10 <100 mg/kg.

REPORTING:
- Final (Day 10): Prepare final reports. Send for key-punching. Send toxicity plot to CCNSC.

WALKER 256 (SUBCUTANEOUS) (CODE 5WA)
ALKYLATING AGENTS ONLY

WA: LD10 determined. Tumor implanted subcutaneously in axillary region of noninbred albino rats. Treatment begun 24 hours after implant. One dose daily for 5 days, by dose response at 4 doses. Animals sacrificed between Days 10 and 14, just before necrosis of tumor begins. Weights of tumors of test animals compared with those of control animals. Results expressed as ratio LD10/ED90.

ANIMALS:	(Protocol 8)
Strain:	Suitable noninbred albino rats.
Weight range:	Male and Female: 90–110 grams.
Sex; source:	One sex and source for each group of test and control animals.
EXPERIMENT SIZE:	(Protocol 9)
General testing:	Six animals per test group (Sec. 9.101).
Control groups:	Number of animals varies according to number of test groups.
TUMOR:	(Protocol 6)
Implant:	Subcutaneously in the axillary region by trocar.
Size of implant:	Average diameter 2–4 mm per fragment.
Propagation:	For line, 11–13 day, donors for test, 11–13 day (Protocol 5).
PRETESTING SCHEDULE:	Determine LD10 in non-tumor-bearing rats (Sec. 1.500).
TESTING SCHEDULE:	(Protocols 3 and 4)
Day 0:	Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
Day 1:	Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 5 at four doses: LD10, LD10/2, LD10/4 LD10/8 (Sec. 1.500). If LD10 > 100 mg/kg test at dose of 200, 100, 50, and 25 mg/kg. Prepare fresh compound each day unless known to be stable.
Day 2:	Recheck cultures. Discontinue testing if contaminated.
Day 10 to 14:	Sacrifice and record wt. of animals and tumors.
EVALUATION:	(Protocol 11) Compute mean animal wt. Day 1, Day 10–14, and T/C of tumor wt. for all groups with more than 65% survivors. Plot data and calculate therapeutic index (TI = LD10/ED 90).
QUALITY CONTROL:	(Protocol 7) Acceptable mean control tumor wt.: 3.0 to 12 grams. Compute S.D. of mean (Sec. 11.003). Positive control limit not established. For NSC-9706, use 0.05 mg/kg. Check control deaths, "no-takes," etc.
REPORTING:	
Initial (Day 1):	Mail initial control and test reports to Documentation Inc.
Final (Day 10–14):	Prepare final control and test reports. Send for key-punching.
CRITERIA:	(Protocol 12) Therapeutic index $\geq$ 4 (Sec. 12.104).

WALKER 256 (INTRAMUSCULAR) (CODE 5WM)

WM: Implanted intramuscularly in thigh of noninbred albino rats. Treatment begun 3 days after implant. One dose daily for 4 days. Animals sacrificed on 7th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: Suitable noninbred albino rats.
- Weight range: Male and Female: 55–65 grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Six animals per test group (Sec. 9.101).
- Assay testing: Four animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Intramuscularly in thigh.
- Size of implant: 0.2 cc of 1–6 dilution of tumor homogenate.
- Propagation: For line, 10–12 day; donors for test, 10–12 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Run positive control on odd-numbered control groups.
- Days 1 and 2: Check cultures for contamination; discard contaminated groups. Determine solubilities; thaw solutions. Prepare materials.
- Day 3: Weigh and randomize animals (Protocol 10). Treat Day 3 through Day 6. Record survivors daily.
- Day 7: Sacrifice and weigh animals. Remove both hind legs at hip joint and weigh individually. Subtract wt. of normal leg from tumor-bearing leg and record as tumor wt.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 3, Day 7, and T/C of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 3–12 grams. Compute S.D. of mean (Sec. 11.003). Positive control limit not established. For NSC-45383, use 0.2 mg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 3): Mail initial control and test reports to Documentation Inc.
- Final (Day 7): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)
- Sequential: (Sec. 12.102)

	Stage 1	Stage 2	Stage 3
Synthetics	≦ .63	≦ .23	≦ .08
Natural products	≦ .60	≦ .22	

- Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.210). Natural products: 2 successive dose-response tests (Sec. 12.204).

DUNNING ASCITES LEUKEMIA (CODE 5DA)

DA: Injected intraperitoneally in Fischer/344 rats. Treatment begun 24 hours after implant. One dose daily for 5 days. Median survival time of test animals compared with that of control animals. Results expressed as percent of control survival time.

ANIMALS: (Protocol 8)
- Strain: Fischer/344 rats.
- Weight range: Male and Female: 90–110 grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Six animals per test group (Sec. 9.101).
- Assay testing: Four animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Intraperitoneal.
- Size of implant: 0.5 ml containing 5×10^6 cells of 7-day ascitic fluid.
- Propagation: 0.5 ml of undiluted ascitic fluid transplanted every 7 days (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 5.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 5: Weigh animals and record.
- Day 30: Sacrifice all survivors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 5, and T/C of median survival time for all groups with more than 65% survivors on Day 5 (Sec. 11.200).

QUALITY CONTROL: (Protocol 7)
Acceptable median survival time: 8–11 days. Positive control limit not established. For NSC-755, use 20 mg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final Day: Prepare final control and test reports on day of last animal death. Send for key-punching.

CRITERIA: (Protocol 12)
- Sequential: (Sec. 12.103)

	Stage 1	Stage 2
Synthetics	$\geqq 1.25$	$\geqq 1.56$
Natural products	$\geqq 1.25$	$\geqq 1.56$

- Confirmation: Synthetics: Dose response with T/C of at least one dose $\geqq 125\%$ (Sec. 12.202). Natural products: Two successive dose-response tests (Sec. 12.204).

DUNNING LEUKEMIA (SOLID) (CODE 5DL)
ALKYLATING AGENTS ONLY

DL: LD10 determined. Tumor implanted subcutaneously in axillary region of Fischer/344 rats. Treatment begun 24 hours after implant. One dose daily for 5 days by dose response at 4 doses. Median survival time of test animals compared with that of control animals. Results expressed as percent of control survival time. Survivors at 30 days are recorded as cures.

ANIMALS: (Protocol 8)
- Strain: Fischer/344 rats.
- Weight range: Male and Female: 90–110 grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Six animals per test group (Sec. 9.101).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region by trocar.
- Size of implant: Average diameter 2–5 mm per fragment.
- Propagation: For line, 10 day; donors for test, 10 day (Protocol 5).

PRETESTING SCHEDULE: Determine LD10 in non-tumor-bearing, noninbred rats (Sec. 1.500).

TESTING SCHEDULE: (Protocols 3 and 4).
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 5 at four doses: LD10, LD10/2, LD10/4, LD10/8 (Sec. 1.500). If LD10 > 100 mg/kg, test at 200, 100, 50, and 25 mg/kg. Prepare fresh compound each day unless known to be stable.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 10: Weigh animals and record.
- Day 30: Sacrifice all survivors and evaluate.

EVALUATION: (Protocol 11)
Compute mean animal weights Day 1, Day 10, and T/C of median survival for all groups with more than 65% survivors on Day 10.

QUALITY CONTROL: (Protocol 7)
Acceptable median control survival time: 12–16 days. Positive control limit not established. For NSC-755, use 20 mg/kg. Check control deaths, "no-takes," etc.

REPORTING: (Protocol 20)
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final Day: Prepare final control and test reports on day of last animal death. Send for key-punching.

CRITERIA: (Protocol 12)
$\geq$ 20% cures at any one dose (Sec. 12.105).

MURPHY-STURM LYMPHOSARCOMA (CODE 5MS)

MS: Implanted intramuscularly in thigh of noninbred albino rats, by using 1 to 6 tumor homogenates for transplant. Treatment begun 24 hours after implant. One dose daily, for 9 days. Animals sacrificed on 10th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: Suitable noninbred albino rats.
- Weight range: Male and Female: 45–55 grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Six animals per test group (Sec. 9.101).
- Assay testing: Four animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Implant homogenates intramuscularly in the thigh.
- Size of implant: 0.2 ml containing appproximately 2×10^6 cells.
- Propagation: For line, 12–14 day; donors for test, 12–14 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4).
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 9.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 10: Sacrifice and weigh animals. Remove both hind legs at hip joint and weigh individually. Subtract weight of normal leg from tumor-bearing leg and record as tumor wt.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 10, and T/C of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 5–20 grams. Compute S.D. of mean control tumor wt. (Sec. 11.003). Positive control limit not established. For NSC-740, use 0.35 mg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final (Day 10): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)

Sequential: (Sec. 12.102)

	Stage 1	Stage 2	Stage 3
Synthetics	$\leqq .63$	$\leqq .23$	$\leqq .08$
Natural products	$\leqq .60$	$\leqq .22$	

Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201).
Natural products: 2 successive dose-response tests (Sec. 12.204).

HUMAN SARCOMA HS1 (CODE 5H1)

H1: Implanted subcutaneously in axillary region of conditioned, noninbred albino rats. Treatment begun 24 hours after implant, hydrocortisone conditioning continued during test. One dose daily for 12 days. Animals sacrificed on 13th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: Suitable noninbred albino rats.
- Weight range: Male and Female: 45–55 grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Ten animals per test group (Sec. 9.102).
- Assay testing: Six animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

CONDITIONING SCHEDULE: X ray: 175 r 1 to 3 days before tumor implant. 3 mg hydrocortisone on Days 0, 2, 4, and 6. Antibiotics administered in drinking water.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region.
- Size of implant: 0.5 ml of 50% tumor brei in balanced salt solution.
- Propagation: For line, 12–14 day; donors for test, 12–14 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: 3 mg hydrocortisone to each rat when X-rayed. Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 12.
- Day 2: Recheck cultures. Discontinue testing if contaminated. Repeat hydrocortisone treatment on Day 2, 4, 6.
- Day 13: Sacrifice and record wt. of animals and tumors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 13, and T/C of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 5–20 grams. Compute S.D. of mean control tumor wt. (Sec. 11.003). Positive control limit not established. For NSC-45383, use 25 μg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final (Day 13): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)
- Sequential: (Sec. 12.102)

	Stage 1	Stage 2	Stage 3
Synthetics	≦ .63	≦ .23	≦ .08
Natural products	≦ .60	≦ .22	

- Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201). Natural products: 2 successive dose-response tests (Sec. 12.204).

HUMAN EPIDERMOID CARCINOMA HEP 3 (CODE 5H3)

H3: Implanted subcutaneously in axillary region of conditioned, noninbred albino rats. Treatment begun 24 hours after implant, hydrocortisone conditioning continued during test. One dose daily for 9 days. Animals sacrificed on 10th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: Suitable noninbred albino rats.
- Weight range: Male and Female: 45–55 grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Six animals per test group (Sec. 9.101).
- Assay testing: Four animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

CONDITIONING SCHEDULE: X ray: 175 r 1 to 3 days before tumor implant. 3 mg hydrocortisone on Days 0, 2, 4, and 6. Antibiotics administered in drinking water.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region.
- Size of implant: 0.5 ml of 50% tumor brei in saline.
- Propagation: For line, 10 day; donors for test, 10 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: 3 mg hydrocortisone to each rat when X-rayed. Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 9.
- Day 2: Recheck cultures. Discontinue testing if contaminated. Repeat hydrocortisone treatment on Days 2, 4, and 6.
- Day 10: Sacrifice and record wt. of animals and tumors.

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 10, and T/C of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 1.5–6 grams. Compute S.D. of mean control tumor wt. (Sec. 11.003). Positive control limit not established. For NSC-45383, use 0.04 mg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final (Day 10): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)

Sequential: (Sec. 12.102)

	Stage 1	Stage 2	Stage 3
Synthetics	$\leqq .63$	$\leqq .23$	$\leqq .08$
Natural products	$\leqq .60$	$\leqq .22$	

Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201).
Natural products: 2 successive dose-response tests (Sec. 12.204).

ADENOCARCINOMA OF THE SMALL BOWEL (CODE 7SB) AND ADENOCARCINOMA OF THE ENDOMETRIUM (CODE 7EN)

SB and EN: Implanted subcutaneously in axillary region of hamsters. Treatment begun 24 hours after implant. One dose daily for 11 days. Animals sacrificed on 12th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: Hamsters 4 to 6 weeks old.
- Weight range: Male and Female: 45–60 grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Ten animals per test group (Sec. 9.102).
- Assay testing: Six animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region by trocar.
- Size of implant: Average wt. 15 mg per fragment.
- Propagation: For line, 10–12 day; donors for test, 10–12 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures; discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 11.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 6: Prepare fresh compound for subsequent testing.
- Day 12: Sacrifice and record wt. of animals and tumors. Examine carcass grossly for metastases and record under "Comments."

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 12, and T/C of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 800–3500 mg. Compute S.D. of mean control tumor wt. (Sec. 11.003). Positive control limit not established. For NSC-26271, use 25 mg/kg. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final (Day 12): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)

Sequential: (Sec. 12.102)

	Stage 1	Stage 2	Stage 3
Synthetics	≦ .63	≦ .23	≦ .08
Natural products	≦ .60	≦ .22	

Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201).
Natural products: 2 successive dose-response tests (Sec. 12.204).

MELANOTIC MELANOMA (CODE 7MM) AND PLASMACYTOMA (CODE 7P1)

MM and P1: Implanted subcutaneously in axillary region of hamsters. Treatment begun 24 hours after implant. One dose daily for 15 days. Animals sacrificed on 16th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: Hamsters 4 to 6 weeks old.
- Weight range: Male and Female: 45–60 grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Ten animals per test group (Sec. 9.102).
- Assay testing: Six animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region by trocar.
- Size of implant: Average wt. 15 mg per fragment.
- Propagation: For line, 16 day; donors for test, 16 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 15.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 6: Prepare fresh compound for subsequent testing.
- Day 16: Sacrifice and record wt. of animals and tumors. Examine carcass grossly for metastases and record under "Comments."

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 16, and T/C of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt. for MM, 2000–4000 mg; for P1, 3000–6000 mg. Compute S.D. of mean control tumor wt. (Sec. 11.003). Positive control to be established. Check control deaths, "no-takes," etc.

REPORTING:
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final (Day 16): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)

Sequential:

(Sec. 12.102)	Stage 1	Stage 2	Stage 3
Synthetics	$\leqq .63$	$\leqq .23$	$\leqq .08$
Natural products	$\leqq .60$	$\leqq .22$	

Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201).
Natural products: 2 successive dose-response tests (Sec. 12.204).

ADENOCARCINOMA OF THE DUODENUM (CODE 7D1)

D1: Implanted subcutaneously in hamster. Treatment begun 24 hours after implant. One dose daily for 7 days. Animals sacrificed on 8th day. Weights of tumors of test animals compared with those of control animals. Results expressed as percent of control growth.

ANIMALS: (Protocol 8)
- Strain: Hamsters 4 to 6 weeks old.
- Weight range: Male and Female: 45–60 grams.
- Sex; source: One sex and source for each group of test and control animals.

EXPERIMENT SIZE: (Protocol 9)
- General testing: Ten animals per test group (Sec. 9.102).
- Assay testing: Six animals per test group (Sec. 9.103).
- Control groups: Number of animals varies according to number of test groups.

TUMOR: (Protocol 6)
- Implant: Subcutaneously in the axillary region.
- Size of implant: Average wt. 15 mg per fragment, or suitable brei.
- Propagation: For line, 8 day; donors for test, 7 or 8 day (Protocol 5).

TESTING SCHEDULE: (Protocols 3 and 4)
- Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100). Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Record survivors daily.
- Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals (Protocol 10). Treat Day 1 through Day 7.
- Day 2: Recheck cultures. Discontinue testing if contaminated.
- Day 8: Sacrifice and record wt. of animals and tumors. Examine carcass grossly for metastases and record under "Comments."

EVALUATION: (Protocol 11)
Compute mean animal wt. Day 1, Day 8, and T/C of tumor wt. for all groups with more than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 750–3000 mg. Compute S.D. of mean (Sec. 11.003). Positive control limit to be established. For NSC-755, use 60 mg/kg/day. Check control deaths, "no-takes," etc.

REPORTING: (Protocol 20)
- Initial (Day 1): Mail initial control and test reports to Documentation Inc.
- Final (Day 8): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)

Sequential: (Sec. 12.102)

	Stage 1	Stage 2	Stage 3
Synthetics	$\leqq .63$	$\leqq .23$	$\leqq .08$
Natural products	$\leqq .60$	$\leqq .22$	

Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201).
Natural products: 2 successive dose-response tests (Sec. 12.204).

HS1—EGG HOST (CODE 8H1)

HS1: Implanted on vascularized area of the chorio-allantoic membrane of 9-day-old embryonated eggs. One dose only on Day 3 or Day 4. Hosts sacrificed on Day 9 or Day 10. Weights of treated tumors are compared with those of control. Results are expressed as percent of control growth.

HOST: (Protocol 13)
9-day fertile eggs.

EXPERIMENT SIZE: (Protocol 9)
General testing: Six eggs per test group (Sec. 9.101).
Assay testing: Four eggs per test group (Sec. 9.103).
Control groups: Number of eggs varies according to number of test groups.

TUMOR: (Protocol 13)
Implant: Implanted on chorio-allantoic membrane of 9-day fertile eggs.
Size of implant: *Average diameter 2–4 mm per fragment.*
Propagation: For line, 10 or 11 day; donors for test, 10 or 11 day.

PRETESTING SCHEDULE: Determine maximum tolerated dose (MTD) in non-tumor-bearing eggs (Sec. 13.400).

TESTING SCHEDULE: (Protocol 3)
Day 0: Implant tumor. Run bacterial cultures (Sec. 7.100).
Day 1: Check cultures; discard contaminated groups.
Day 2: Recheck cultures. Determine solubilities; thaw solutions. Prepare materials. Run positive control on odd-numbered control groups. Randomize eggs (Protocol 10).
Day 3 or 4: Inject drug into yolk sac of tumor-bearing eggs. Record survivors daily for 6 days.
Day 9 or 10: Sacrifice hosts and record wt. of embryos and tumors.

EVALUATION: (Protocol 11)
Compute mean embryo and tumor wt. and T/C of tumor wt. for all treated groups having greater than 65% survivors.

QUALITY CONTROL: (Protocol 7)
Acceptable mean control tumor wt.: 500–2000 mg. Compute S.D. of mean (Sec. 11.003). Positive control limit not established. For NSC-26271, use 0.5 mg/egg. Check control deaths, "no-takes," etc.

REPORTING:
Initial (Day 3): Mail initial control and test reports to Documentation Inc.
Final (Day 10): Prepare final control and test reports. Send for key-punching.

CRITERIA: (Protocol 12)
Sequential: (Sec. 12.102)

	Stage 1	Stage 2	Stage 3
Synthetics	$\leqq .63$	$\leqq .23$	$\leqq .08$
Natural products	$\leqq .60$	$\leqq .22$	

Confirmation: Synthetics: 3 successive tests at sequential dose (Sec. 12.201).
Natural products: 2 successive dose-response tests (Sec. 12.204).

CELL CULTURE (CODE 9KB)

KB: KB cells cultivated on Eagle's basal medium plus 10% serum. Stock cells are fed 24 hours before use on test. Test material added on Day 0 or Day 1. Results are expressed as dose that inhibits growth to 50% of control growth, 3 days after drug addition.

EXPERIMENT SIZE: (Protocol 14)

General testing: Three to five dose levels per material. Two tubes per dose level.

Control groups: Number to vary according to number of test groups (n), according to the formula: $2\sqrt{n}$. Determine baseline protein according to method of Oyama and Eagle.

TESTING SCHEDULE:

Day 0: Dilute stock cells to 10–20 μg/ml in complete media. Add cells to tubes and add test material simultaneously or on Day 1. Total volume is approximately 4 ml (Sec. 14.003). Run positive control on odd-numbered control groups.

Day 1: If 24-hour culture used, refeed and add test material. Determine protein values of baseline tubes.

Day 3: Protein analysis of test, control, and at least 3 protein standard and media blank tubes (Sec. 14.300).

Day 4: If 24-hour cultures used, protein analysis as prescribed for Day 3.

DOSAGE: (Protocols 3 and 4)

Test synthetics and plant products by weight (W) at 100, 10, and 1 μg/ml. Test crude fermentation products by dilution (D) at 1:10, 1:100, and 1:1000. Test dried or crystalline fermentation products by weight at appropriate concentrations. Any material which does not reach an end point at these levels is to be retested at lower concentrations (Secs. 14.100, 14.500). All additional tests carried out at five dose levels at 0.3 log intervals.

QUALITY CONTROL: Control tubes must show growth of at least 6 times that of baselines (Sec. 14.400). Positive control limit not established. Use NSC-755.

REPORTING:

Final (Day 3 or 4): Mail test and control screening reports to Documentation Inc.

CRITERIA:

Sequential: (Sec. 12.106)

	Stage 1	Stage 2
Synthetics	ED50 ≦ 6μg/ml	ED50 ≦ 4 μg/ml
Plant extracts	ED50 ≦ 30 μg/ml	ED50 ≦ 20 μg/ml
Fermentation products	ED50 > 1:100 dilution	ED50 > 1:100 dilution

Confirmation: (Sec. 12.203)

Synthetics	ED50 ≦ 4 μg/ml
Plant extracts	ED50 ≦ 20 μg/ml
Fermentation products	ED50 > 1:500 if known classes of cytoxic agents are excluded.